

Female reproductive conflict and prenatal investment



Submitted by

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Emma L. Inzani

Summary

With limiting resources and large demands for resources to breed, this can result in reproductive conflict between individuals of the same sex. Female reproductive competition to produce as many offspring as possible, can come in many forms but the intensity is often underestimated as their methods of competing can be quite subtle. In cooperative species, with high local competition and reproductive skew between females, competition can be more obvious with aggression that can lead to eviction of a female from the group or infanticide. However, females may be able to try to outcompete each other by investing more in their offspring prenatally, giving their offspring a head start. In this thesis, I investigate what influences female prenatal investment and its consequences in a wild population of banded mongooses (*Mungos mungo*). This work was conducted as part of a long-term project studying a wild cooperative mammal; with valuable data on individuals' prenatal investment available. Firstly, (Chapter 1) I discover that female investment in fetus size is influenced by female reproductive competition and the level of resources available, which alters the intensity of the conflict experienced. However, I found no advantages or disadvantages to this increase in investment on the short-term survival or weight of their pups. In Chapter 2, I found that when conditions are bad or being a younger and more subordinate female increases the probability of reproductive failure (abortion). Further research is needed to find out if this is an adaptive strategy to conserve energy when experiencing high levels of competition.

Overall, this work helps to contribute to our understanding of how wild mammals might be subtly able to alter their prenatal investment, with regards to their competitive environment, and how this might affect their life history strategies.

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Author's declaration

All chapters were written by Emma L. Inzani with comments provided by Dr. Emma Vitikainen and Professor Michael Cant. Additional comments were given on Chapter 2 provided by the co-authors on publication from this chapter.

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All research procedures received prior approval from Uganda Wildlife Authority and Uganda National Council for Science and Technology, and adhered to the Guidelines for the Treatment of Animals in Behavioural Research and Teaching, published by the Association for the Study of Animal Behaviour. All research was approved by the Ethical Review Committee of the University of Exeter.

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Publications

At time of printing **Chapter 2** has been published in *Nature scientific reports* exactly as it appears here.

Female reproductive competition explains variation in prenatal investment in wild banded mongooses

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Chapter 1: General Introduction

Intrasexual competition-differences male and female

Intrasexual competition is a major force shaping the behaviour and morphology of sexual organisms. Given finite resource, individuals have to compete for space, food and reproduction. Conflict over reproduction among males is often conspicuous, and involves obvious displays of aggression and the development of exaggerated secondary sexual characteristics that increase a male's chances of obtaining matings (Anderson 1994; Clutton-Brock *et al* 2007). For females, by contrast, reproductive success tends not to be limited by number of mates, but rather by access to resources. Females have traditionally been considered to compete less strongly than males for opportunities to breed. However, in systems where there is high reproductive skew among females, such as meerkats (*Suricata suricatta*), common marmoset (*Callithrix jacchus*) and naked mole rats (*Heterocephalus glaber*), competition between females can be intense and variance in reproductive success can be greater than that seen in males (Clutton-Brock 2009; Lacey & Sherman 1997; Saltzman *et al* 2009; Young *et al* 2006).

Female reproductive competition

Female reproductive competition occurs due to a large variance in resource acquisition between females, which strongly influences female fitness. The resources in question include food, space, mates and helpers to assist with postnatal care (Bro-Jørgensen 2002; Thompson *et al* 2007; Creel & Wasser

1991). Intrasexual selection should favour females that can gather and allocate as much resources as possible towards reproduction (Clutton-Brock 2006).

Like male- male competition females can also display secondary sexually selected traits as an indicator of quality or competitive ability (Clutton-Brock et al 2006; Clutton-Brock 2009). An example is the female *Eclectus* parrots (*Eclectus roratus*) which has vividly coloured ornamental plumage that is more striking than the males. Females compete for nest sites in trees which are a rare and limiting factor for their reproductive effort. This intense competition for nest cavities has led to the sexual selection of bright ornamental plumage by which females advertise their own quality and assess the quality of rivals. In this species females are freed from the constraint of having to remain cryptic as they nest in dark cavities (Heinsohn *et al* 2005).

If resources are defendable females can exclude their competitors by defending a territory in which they can monopolise the resources needed to breed. Female bay wrens (*Thryothorus nigricapillus*) defend their territories through vocalisations and duets with their mate. Through a series of removal experiments it was determined that females singing to defend their territory from female competitors (Levin 1996; Langmore 1998). House mice (*Mus domesticus*) defend resource rich territories against individuals of the same sex, regardless of individual size/quality (Gray *et al* 2002).

In gregarious or group-living species, females experience particularly high levels of local resource competition. In these systems females often form dominance hierarchies based on their assessed relative quality or competitive

ability (Rowell 1974; Kaufmann 1983). High ranking females have superior access to resources and often feed more efficiently. In reindeer (*Rangifer tarandus*), for example, dominant females outcompete subordinate females for high quality foraging areas, and as a consequence produce larger calves and have shorter interbirth intervals than subordinate females (Holland *et al* 2004). Similarly, in female hyenas (*Crocuta crocuta*) higher ranked females outcompete subordinates when resources are scarce (Holekamp *et al* 1996).

In cooperatively breeding species, in which helpers routinely help to rear offspring that are not their own, reproductive competition among females has led to the evolution of a diverse array of competitive strategies. Some of these strategies involve intense aggression. Dominant females can inhibit subordinate female reproduction by evicting them from the group or by killing their offspring (Cant *et al* 2014, Clutton-Brock *et al* 2010; Hackländer *et al* 2003; Lacey & Sherman 1997). In meerkats (*Suricata suricatta*), for example, dominant females evict pregnant subordinate females from the group if they become pregnant. Eviction involves intense aggression and often results in pregnant subordinates spontaneously aborting their litter (Clutton-Brock *et al* 2001; Young *et al* 2006). However, eviction behaviour can also inflict costs on dominant females. In banded mongooses, dominant females who evict subordinates suffer a reduction in the weight and survival of their own pups (Bell *et al* 2011; Bell *et al* 2014). Dominant females can also postnatally compete through infanticide (Hodge *et al* 2011).

Females can also compete through more subtle using threats of eviction or infanticide to deter subordinate breeding. In common marmosets (*Callithrix*

jacchus) and meerkats dominants will evict pregnant subordinates or infanticide in order to avoid competition (Young *et al* 2006; Saltzman *et al* 2009). However, through frequent and low level aggression towards subordinates as a threat, it has been found to elevate subordinates stress response and thus inhibit the subordinate from reproduction by reducing female condition (O’Riain *et al* 2000; Saltzman *et al* 2009). Currently, there is very little known about these subtle means of reproductive competition.

Reproductive allocation and trade-offs

Life history theory suggests that key processes such as growth, maturity, reproduction and survival throughout an organism’s life cycle are optimized to produce the maximum number of offspring to survive and reproduce (Stearns 1992). Since organisms have a finite amount of energy to be invested into different functions, there inevitably arises trade-offs between life history traits in resource allocation (Stearns 1989). For example, parents face a trade-off between offspring size and number (Lack 1947; Smith & Fretwell 1975). The optimal allocation to size vs number of offspring depends on the selective pressures on different traits that influence reproductive success, such as female condition and size, level of parental care needed and the environmental conditions (Walker *et al* 2008). Life history tradeoffs and optimal allocation decisions may also vary across the lifespan. For example, the investment in quality and quantity of the offspring produced in red deer (*Cervus elaphus*) increased with age initially, but later declined with age due to the impact of previous reproductive effort decreasing the levels of resources available for current and future reproductive event (Nussey *et al* 2006).

In mammals, females may face complicated allocation decisions and trade-offs due to gestation followed by a long period of offspring dependency. The most costly part of rearing offspring is lactation (Clutton-Brock 1989): the energetic costs of producing and gestating fetuses are small compared to the resources needed for milk production. Even aborting an existing pregnancy may therefore be an adaptive strategy, if chances of successfully rearing a litter are small, to avoid costs of lactation and enable the female to save resources to a future breeding attempt. In Gelada baboons (*Theropithecus gelada*), females will abort or reabsorb fetuses in the presence of a new unknown male ('The Bruce effect') where males are likely to commit infanticide in order to induce females to come into oestrus (Bruce 1959; Roberts *et al* 2012). This appears to be an adaptive strategy in conserving a female's time and resources to raise future offspring with better survival probabilities, than a current litter likely to fail (Stearns 1989).

Cooperation between females changes the competitive environment and may have profound impacts on reproductive allocation decisions. In cooperative breeders, helpers can increase overall reproductive success by providing additional resources, which may in turn impact optimal patterns of maternal investment (Mares *et al* 2012). For example, in many bird species, offspring have increased survival when they receive extra-parental provisioning and care from more helpers (up to an optimum group size due increased competition; Komdeur 1994). In some species this extra care allows breeding females to reduce their investment in egg size without a corresponding reduction in offspring mortality (Brouwer *et al* 2012). In sociable weavers helpers contribute to increased offspring fledging success in years of adverse conditions (high

rainfall) and when part of a large colony are likely to experience higher competition for resources (Covas *et al* 2008). In superb starlings, having helpers reduced the variation in reproductive success during years of good conditions (Rubenstein 2011). Having helpers can be a strategy to help buffer unpredictable environmental conditions that could impact the optimal reproductive investment required to breed successfully; this allowing maintenance of a constant reproductive success.

Maternal effects and PARs

There is much evidence that the level of maternal investment can have profound effects on offspring fitness. For example, in Italian tree-frogs (*Hyla intermedia*) increased female size allows females to produce more and larger eggs that develop into larger, competitively superior tadpoles (Cadeddu & Castellano 2012). Similarly, in zebra finches (*Taeniopygia guttata*) mothers that have high stress produce offspring that have a reduced lifespan (Gorman & Nager 2004; Tessler *et al* 2014; Haussmann *et al* 2012; Heidinger 2012). These studies complement parallel findings in biomedical research, which shows that maternal condition has profound impacts on development and adult health (the 'foetal programming' hypothesis; Langley-Evans 2004; Godfrey & Barker 2000).

Given these transgenerational or maternal effects on offspring development and life history, there is much interest in the possibility that females may maximise their reproductive success through adjustment of the early life environment experienced by their offspring (Mousseau & Fox 1998). The Predictive Adaptive Response (PAR) hypothesis suggests that mothers adjust the

developmental trajectory of their offspring to match the anticipated postnatal or even adult environment that the offspring will encounter (Gluckman & Hanson 2004; Bateson *et al* 2014). Experimental evidence in support of this hypothesis comes from studies of red squirrels (*Tamiasciurus hudsonicus*) In this species, experimental simulation of high density environments results in high levels of glucocorticoids in mothers, which in turn appears to prime offspring to compete more intensely after birth (Dantzer *et al* 2013) Another example of a PAR in the fall field cricket (*Gryllus pennsylvanicus*) where females exposed to a predator and found that offspring were sensitive to a cue of a predator than those from naïve mothers. However, the PAR hypothesis remains controversial. For example, it has been argued that PARs are unlikely to evolve because long term forecasts of environmental conditions from early life are inherently unreliable (Wells 2007a; Rickard & Lummaa 2007). Any predictive adaptive responses are likely to evolve to match offspring to the immediate post-natal environment, rather than the anticipated adult environment (Rickard & Lummaa 2007). Cooperative breeders are prime candidates to evolve these 'post-natal' PARs because the quality of the postnatal environment is largely determined by the number of breeders competing for reproduction and the number of helpers available to offspring. These features of social groups remain stable over the course of offspring development, from gestation to nutritional independence, so are highly predictable.

In this thesis I investigate strategies of female reproductive competition in a cooperatively breeding mammal, the banded mongoose *Mungos mungo*. Specifically, I have two aims. First, I test whether mothers adjust prenatal investment according to the anticipated post-natal competitive environment.

Second, I test whether patterns of abortion reflect strategic decisions to terminate reproductive investment in the face of reproductive competition. Below I introduce the banded mongoose study system and the basic methods I used to pursue these research aims.

Study system: The banded mongoose (*Mungos mungo*)

Banded mongooses are small diurnal carnivores that live in mixed-sex groups ranging in size 8-40 (Cant 2000; Cant *et al* 2013). They are cooperative breeders, with a core group of 1-5 dominant (dominance correlates with age) females plus any younger subordinate females (females are first reproductively active from about 10 months old). Males have an age based hierarchy for breeding (typically first acquiring paternity from 3 years of age onwards) with usually the oldest and most dominant top 3 males siring most of the offspring conceived within a group (Cant *et al* 2013; Cant *et al* 2016; Nichols *et al* 2010). A group will reproduce 4 to 5 times a year, with females synchronously entering oestrus from 7-10 days after giving birth (within a single group but not between groups). This is to help avoid infanticide by the dominant females if any subordinates gave birth before as larger pups outcompete smaller pups, so any pups born before the dominant females' would give an advantage (Cant 2000; Cant *et al* 2010; Cant *et al* 2014; Gilchrist 2006; Gilchrist 2008; Hodge *et al* 2009; Hodge *et al* 2011). Females gestate for 60 days, giving birth to 4 pups on average per female, with 64% of females within a group giving birth on the same day/night (Cant 2000; Hodge *et al* 2011). The dominant males will 'mate-guard' the females during oestrus to try and defend their paternity of the pups

over the other more subordinate males within a group who will also try to mate with the females if they can (Cant 2000).

All adult group members participate in helping to rear the communal litter. All members will help with rearing the litter through babysitting (1-5 individuals forego foraging to guard the den while the pups are still in it), which strongly influences whether the litter is likely to survive to emergence (Cant 2003; Marshal *et al* 2016). Once they have emerged from the den, offspring form close one-to-one bonds with individual adults (termed 'escorts'). Escorts provide prey items (females will also allolactate) and protect the pups until they reach nutritional independence at around 3 months (Cant 2000; Gilchrist & Russell 2007). Competition between the pups for an escort is strong. Pups that are successful in gaining access to an escort show faster growth and higher survival to 3 months (Gilchrist 2008; Hodge 2005).

There is almost no reproductive suppression in this species, and most females breed regularly from the age of 1 year. When the number of adult females grows large, older, socially dominant females evict younger subordinate females from the group en masse. Evictions start suddenly and involve high levels of aggression directed towards multiple individuals. These individuals are repeatedly attacked for one or more days until they leave the group (Cant *et al* 2010; Gilchrist 2006; Thompson *et al* 2016). In around half of evictions evictees are eventually permitted to rejoin the group. In the remaining cases, evicted subordinates split into single sex groups and disperse out of the main group's territory to found their own group.

Study site and data collection

Banded mongooses can be found throughout sub-Saharan Africa in mixed scrub savannah habitat.

Our study population is located on the Mweya Peninsula on the edge of Lake Edward in Queen Elizabeth National Park, Uganda (0°12'S, 27°54'E). The peninsula is split into two halves (upper and lower) by a 40m high slope, the predominant habitat is savannah grassland with *Euphorbia candelabra* trees, *Euphorbia candelabrum* scattered throughout and denser scrub patches consisting of woolly caper bush, *Capparis tormentosa*, and needle bush, *Azima tetracantha* (Cant 2000). Large herbivores that share the peninsula include African elephant, *Loxodonta Africana*, bushbuck, *Tragelaphus spp.*, Cape buffalo, *Syncerus caffer*, giant forest hog, *Hylochoerus meinertzhageni*, hippopotamus, *Hippopotamus amphibious*, warthog, *Phacochoerus africanus*, and waterbuck, *Kobus ellipsiprymnus*. Common large predators including leopard, *Panthera pardus*, African lion, *Parthera leo* and spotted hyena, *Crocuta crocuta* are also observed. The equatorial climate is relatively constant, with little fluctuation in temperature but has two dry periods with low rainfall in January-February and June-July (Marshall *et al* 2016).

All individuals in the population are captured approximately every 3 months from about 7/8 weeks of age until they die or leave the population to give them unique identifiable markings so they can be identified in the field; as well as gathering biometrics, blood and tissue samples for genetic analysis. Individuals are trapped using a Tomahawk box trap (67 x 23 x 23 cm; Tomahawk Live Trap Co., Tomahawk, USA) and anaesthetised using either ketamine or isoflurane (for further details of the trapping procedure, see Hodge 2007; Jordan

et al 2010). DNA from the tissue samples was used to construct a pedigree using 43 polymorphic microsatellite markers to estimate relatedness and assign parentage (further details of the process are in Sanderson *et al* 2015). When females are pregnant, the number of fetuses was counted under anaesthesia by palpating the abdomen, and a cross-sectional ultrasound scan of each fetus was obtained using an ultrasound scanner (SIUI CTS-900V, UK) and ultrasound gel (Anagel, UK). Trapping females within the last few weeks of pregnancy was avoided and most trapping was conducted 3-4 weeks after oestrus. Previous study has shown no adverse effects of trapping and palpating pregnant females (Gilchrist 2004). Individuals are also habituated to step onto electronic scales with a weak dilute milk solution used as a reward to gain accurate individual weights pre and post foraging and overnight weight loss, without capture which occurs once a week.

To track each social group so that observers can collect life history and behavioural data, each group has one or two individuals that wears a VHF radio collar (Sirtrack Ltd., Havelock North, New Zealand) with a 20cm whip antenna (Biotrack Ltd., UK) to aid location of the groups. All individuals within the study population are habituated to allow an observer within 5m so that detailed behavioural data to be collected. Groups are visited every 1 to 3 days for observational data collection, which is increased to daily when females are in oestrus, expected to give birth, there are dependent pups or if an eviction event has occurred. In a morning, observers will locate a group, before 07:00 as the mongooses emerge from their den, and then follow them foraging till about midday; as the mongooses retire to their dens to avoid the hottest temperatures of the day. At about 16:00, groups are relocated as they start their afternoon

foraging session until 19:00 when they will return to their dens to sleep. The observational/behavioural data is collected through a combination of *ad libitum*, scan sampling, and focal observations; which is recorded on Psion LZ-64 handheld data loggers (Psion Teklogix Inc., Ontario, Canada).

Thesis outline

Although there have been many studies on the variation between individuals in cooperative breeding species in reproductive success, the individual variation in offspring size and number is still poorly understood. This study aim is to examine prenatal investment in a wild mammal in this thesis; my aim was to investigate female reproductive conflict and prenatal investment using the banded mongoose as a model system.

Chapter 2 examines the variation seen in prenatal investment in fetus size and number and whether this is a means for females to compete reproductively. (1) whether mothers adjust prenatal investment in response to reproductive competition, and (2) the consequences of variation in prenatal investment for mothers and offspring.

Chapter 3 investigates the spontaneous abortions of females within a group breeding attempt and whether this in a response to reproductive competition between females. (1) at a group level, under conditions when breeding asynchrony and/or number of competitors reduce the probability of successfully rearing offspring to independence. (2) on individual level depending on female

condition/status, with females in lower condition or of lower social-rank less likely to be able gestate to term.

Chapter 4 is a synthesis of the general findings of chapters 2 and 3.

Chapter 2: Female reproductive competition explains variation in prenatal investment in wild banded mongooses

Abstract

Female intrasexual competition is intense in cooperatively breeding species where offspring compete locally for resources and helpers. In mammals, females have been proposed to adjust prenatal investment according to the intensity of competition in the postnatal environment (a form of 'predictive adaptive response'; PAR). We carried out a test of this hypothesis using ultrasound scanning of wild female banded mongooses in Uganda. In this species multiple females give birth together to a communal litter, and all females breed regularly from one year old. Total prenatal investment (size times the number of fetuses) increased with the number of potential female breeders in the group. This relationship was driven by fetus size rather than number. The response to competition was particularly strong in low weight females and when ecological conditions were poor. Increased prenatal investment did not trade off against maternal survival. In fact we found the opposite relationship: females with greater levels of prenatal investment had elevated post-natal maternal survival. Our results support the hypothesis that mammalian prenatal development is responsive to the intensity of postnatal competition. Understanding whether these responses are adaptive requires information on the long-term consequences of prenatal investment for offspring fitness.

Key words: Female reproductive conflict; prenatal investment; cooperative breeding; predictive adaptive responses; intrasexual competition

Introduction

Intrasexual competition is usually most severe among males, because males generally have higher variance in reproductive success than females (Andersson 1994). This is manifested through conspicuous traits such as aggression and weaponry (Clutton-Brock 2007). In cooperatively breeding species, female competition for reproduction is also intense, leading to overt and sometimes aggressive competition (Clutton-Brock & Huchard 2013). Because the cost of producing young is higher for females compared to males, theory suggests females will often resolve conflict without recourse to overt violence, for example, through the use of signals or threats (Cant & Young 2013).

Recently, it has been suggested that females may compete over reproduction via maternal effects on offspring growth. In hyenas (*Crocuta crocuta*) and red squirrels (*Tamiasciurus hudsonicus*), for example, there is evidence that mothers prime their offspring to face competitive social environments through hormonal signaling (androgens or glucocorticoids [GCs], Dloniak *et al* 2006; Danzter *et al* 2013). Experimental manipulations of population density in other taxa have also shown that offspring size is increased in response to adverse conditions (increased competition) rather than producing more offspring (Allen

et al 2008; Burns *et al* 1995; Cleuvers *et al* 1997; Kindvater *et al* 2014; Meylan *et al* 2007). These effects can be interpreted as a form of 'predictive adaptive response' (PAR), whereby mothers (or, potentially, offspring themselves) are hypothesized to adjust early life developmental trajectories to maximize the fitness of their offspring with the environment experienced post-natally or in later life (Bateson *et al* 2014; Gluckman *et al* 2004; Rickard, & Lummaa; Wells *et al* 2007). However, no study to date has directly tested whether mothers adjust prenatal investment according to the social environment and intensity of reproductive competition in a wild mammal.

We carried out this test in a wild cooperatively breeding mammal, the banded mongoose (*Mungos mungo*) (Cant *et al* 2012). Banded mongooses are small diurnal carnivores which live in stable groups of ~20 adults plus pups. Multiple females (mean= 3.5 females, range 1 to 13) give birth together in each breeding attempt, usually on the same day. Groups breed on average four times per year, experiencing considerable variation in environmental conditions (i.e. rainfall) which is strongly linked to invertebrate prey abundance (Rood 1975; Cant *et al* 2013). Females compete postnatally using infanticide, but can escape infanticide through birth synchrony (Cant *et al* 2014). Offspring compete for access to lactating females and helpers (called "escorts") who provision and protect pups after they emerge from the den. There is also evidence of prenatal maternal impacts on offspring competitiveness: mothers that are heavier at conception produce larger pups which have competitive advantage when competing for alloparental care; increasing pup survival (Hodge *et al* 2009).

We carried out ultrasound scans on 59 breeding females from 8 groups of banded mongooses to test (1) whether mothers adjust prenatal investment in response to reproductive competition, and (2) the consequences of variation in prenatal investment for mothers and offspring.

Methods

Study site

We studied a population of banded mongooses living on and around Mweya Peninsula, Queen Elizabeth National Park (QENP), Uganda (0°12'S, 27°54'E) between May 2000 and November 2013. For a detailed description of the climate, habitat and the population see Cant *et al.* 2013¹⁸. Rainfall data was provided by Uganda Institute of Ecology Meteorological Station and, later, using a rain gauge.

Study population

All individuals in the population are known and individually marked with either colour-coded collars (7 g) or unique shave patterns (for details of trapping protocol and anesthesia are given elsewhere; Ketamine, Hodge 2007; Isoflurane Jordan *et al* 2010). The identity of breeding females was determined from changes in body shape, ultrasound scans and palpation (Cant 2000; Gilchrist *et al* 2004). Each group was visited daily to determine accurate parturition dates. Since parturition can be determined precisely but conception cannot, we calculated the age of fetuses retrospectively assuming an average 60 day gestation (the mean period between peak mate guarding and birth; Cant 2000). Group size and the number of females were counted as the total number

of individuals or females over 1 year old in each group for each communal litter. Individuals are habituated to step onto electronic scales to determine an accurate weight which allows regular weighing events without capture. Female weight at the time of conception was calculated using the closest weighing event prior (± 10 days from conception) to the estimated conception date; if possible weights for all females within the same group came from the same weighing event.

Measuring fetus size and number

Number of fetuses was counted under anesthesia by palpating the abdomen, and a cross-sectional ultrasound scan of each fetus was obtained using an ultrasound scanner (SIUI CTS-900V, UK) and ultrasound gel (Anagel, UK). Trapping females within the last few weeks of pregnancy was avoided and most trapping was conducted 3-4 weeks after oestrus. Previous study has shown no adverse effects of trapping and palpating pregnant females (Gilchrist *et al* 2004). The age of the fetus at the time of the ultrasound scan was calculated retrospectively from the litter birth date and the scan date, assuming a gestation length of 60 days (average female gestation length Cant 2000).

We used the cross-sectional area (mm^2) of each fetus as measured from the ultrasound images as an estimate of fetus size. Fetuses were measured on average at 30 ± 7 (mean \pm sd) days post conception when they are still roughly spherical in shape to minimize noise arising from different angles of the scan cross-section. The outline of a fetus was identified by the black pixilation of the fluid-filled amniotic sac and the white pixilation of the womb tissue and the amniotic sac membrane around the fetus. The mean of two perpendicular

measurements of the diameter were taken using the computer software Image J (1.47c, Rasband 2014) and used to calculate the elliptical area of the fetus (see Figure 1).

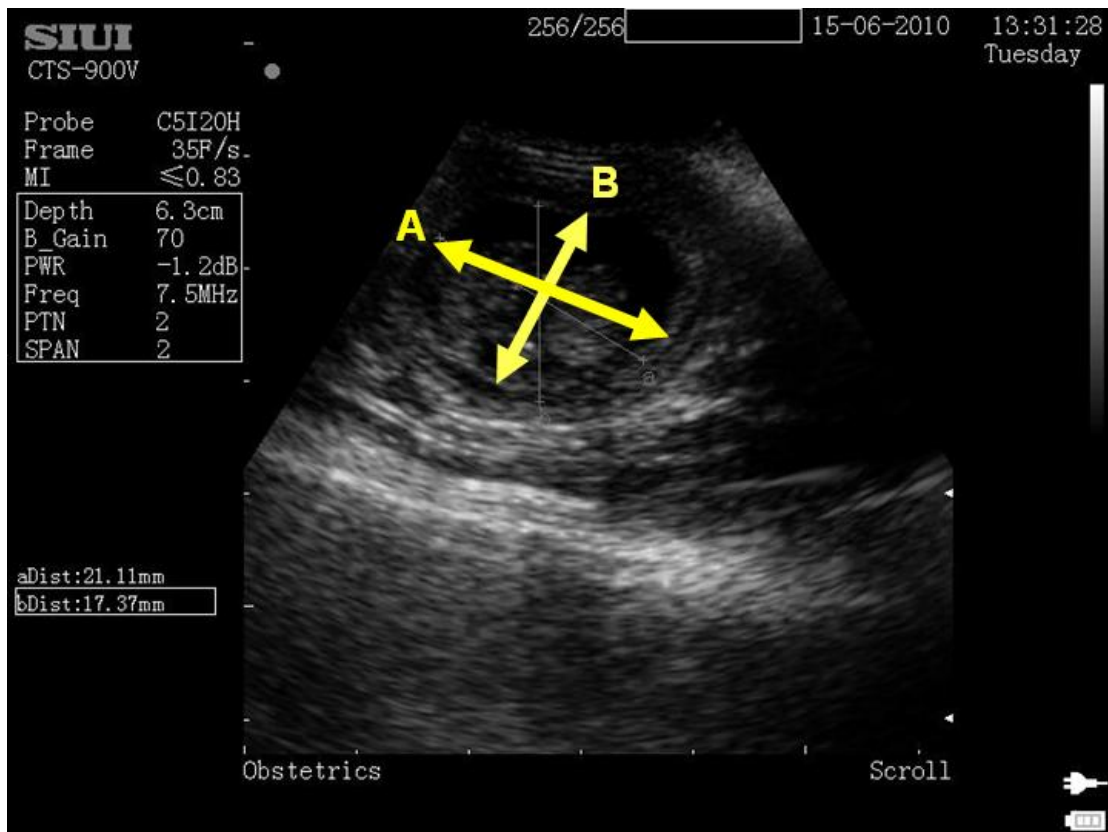


Figure 1. Cross-sectional ultrasound scan of individual fetus with 2 perpendicular measurements A and B used to calculate the cross-sectional area ($A/2 \times B/2 \times \pi$).

Statistics

We analyzed fetus sizes and the number of fetuses using general linear mixed models (LMMs) and generalized linear mixed models (GLMMs) in R version 3.1.0 using lme4 package R1.1-6 (Bates *et al* 2012; R development Core team 2014). GLMMs had either a poisson error structure with log-link function or binomial error structure with logit link function. Female, litter and group identities

were included as random factors in analyses to account for the repeated sampling. Fixed terms included were female weight at conception, female age (months), number of adult females present in the group, group size and the total rainfall during gestation (ml). Because groups were trapped at different stages of pregnancy, fetus age (days) was included as a covariate when analyzing fetus size. Correlations between variables fitted in the same models as fixed effects were lower than the levels indicated by Freckleton(2011) to cause model fitting issues such as variance inflation in effect estimates (max $r = 0.48$). We obtained a minimal model via sequential removal of least significant factors, starting with 2-way interactions. Each factor was then added back into the minimum model in order to confirm removal was not contingent on the order of removal (Crawley 2007).

To investigate if mothers adjust their prenatal investment in response to reproductive competition we estimated total prenatal investment by multiplying the average fetus size by the number of fetuses carried for each pregnancy. Variation in prenatal investment could be due to individual female adjustment in response to competition (a within-individual effect) or be the result of consistent differences between individuals. We tested the relative importance of within- versus between-individual effects using the method described by van de Pol & Wright (2009), which separates out the effect sizes in the fitted model attributable to variation within versus between individuals. To test the consequences of variation in prenatal investment for mothers and offspring we focused on pup survival to 3 months (y/n) using logistic regression, and pup weight (controlled age at capture <90 days) as well as female reproductive effort and survival. Maternity assignments for pups were based on 43

microsatellite loci as described in Sanderson *et al* (2015). As individual fetus scans cannot be matched to pups an average fetus size was used in these analyses. Relative fetus size was calculated as the average fetus size in each female's litter relative to average fetus size for all females within a breeding attempt. We tested whether prenatal investment predicted female participation in the next group litter (y/n) using a GLMM with binomial errors. We tested whether there was a trade-off between current investment in reproduction and female survival using Cox regression with backward selection of terms (Wald Chi-square). This analysis included total group size, number of females, and the average fetus size and number of fetuses as predictors, and to avoid repeat sampling used only the last reproductive event on record for each female. This analysis was conducted in SPSS 21.0.0.0 (IBM Corp. 2012).

Results

(1) Do mothers adjust prenatal investment in response to reproductive competition?

The total prenatal investment (fetus size x number of fetuses carried) of females increased with the number of other adult females in the group during pregnancy, and with a female's weight at conception (LMM, number of females, $\chi^2_1=5.65$, $N=142$, $P=0.017$, female weight: (LMM, , $\chi^2_1=12.60$, $N=142$, $P<0.001$). This relationship was driven by fetus size rather than number: mean fetus size increased with the number of females in the group; increased more steeply in lighter females, and in breeding attempts featuring lower rainfall (LMM, 2 way interaction of female number with: weight, $\chi^2_1=4.23$, $N=360$ scans, $P=0.040$; rainfall, $\chi^2_1=4.91$, $N=360$, $P=0.027$; Figure 2). Neither total

group size nor female age influenced fetus size (see Supplementary Table S1). Within-female variation was a better predictor of fetal size in response to reproductive competition than between-female variation (LMM, within-female variation, $\chi^2_1=4.51$, $N=360$, $P=0.034$, between-female variation, $\chi^2_1=3.38$, $N=360$, $P=0.066$; Supplementary Table S2). The number of fetuses was only influenced by female age, peaking at 4 years of age before declining (GLMM poisson, $\chi^2_1=10.36$, $N=361$, $P=0.001$). There was no significant relationship between fetus size and the number of fetuses (LMM, $\chi^2_1=1.03$, $N=581$, $P=0.31$). Thus individual females produced larger fetuses, but no fewer of them, when faced with competition from other female breeders.

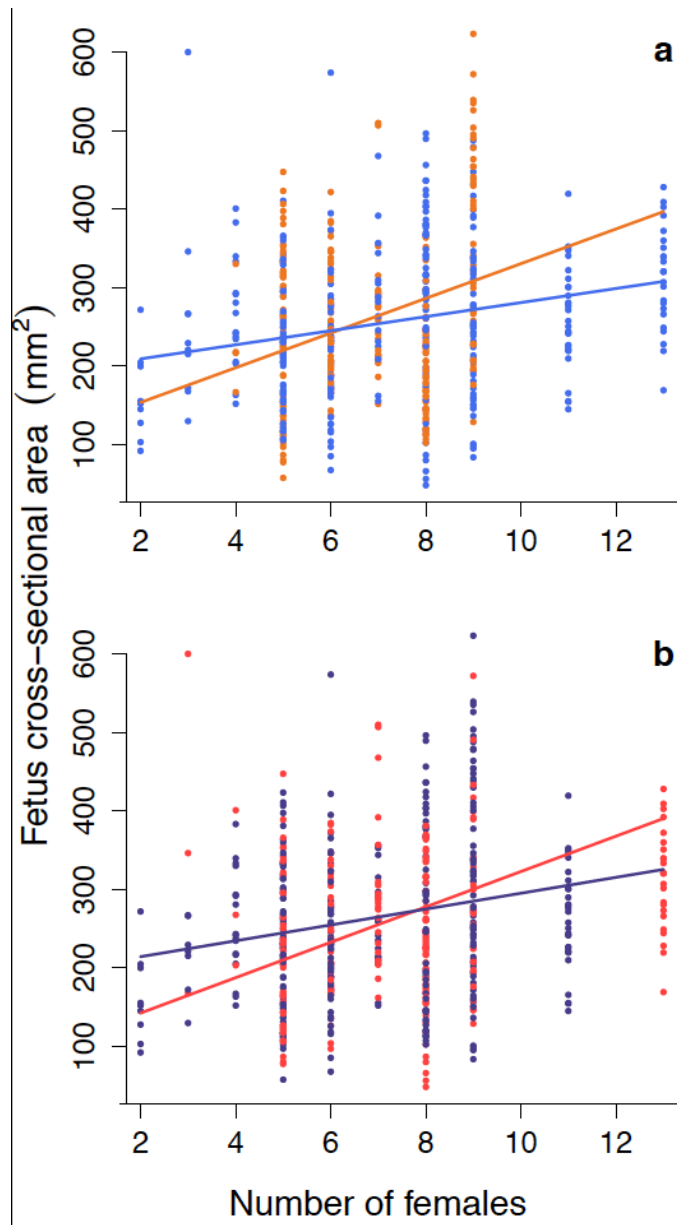


Figure 2. Variation in prenatal investment as a function of the number of adult females in the group at conception. (a) Fetus cross-sectional area increases more sharply when rainfall is low (orange line) compared to high (light blue line); (b) Lighter females (red line) show the steepest increase in fetus size with female number compared to heavier females (dark blue line). Female weight (mean \pm sd = 1447 \pm 201g) and rainfall (mean \pm sd = 128.3 \pm 40.9ml) are continuous variables that have been categorized for illustrative purposes using the 25% and 75% quartiles.

(2) What are the consequences of variation in prenatal investment for mothers and offspring?

Female reproductive success (number of assigned pups at emergence) increased with the number of fetuses during gestation, (GLMM poisson, $\chi^2_1 = 5.44$, $N = 153$ females, $P = 0.02$; Supplementary Table S3). However, larger fetuses did not translate into a greater number of assigned pups (GLMM poisson, $\chi^2_1 = 0.76$, $N = 151$ pups, $P = 0.38$). Fetus size also did not influence pup weight at 3 months (LMM, $\chi^2_1 = 0.37$, $N = 115$ pups, $P = 0.54$; Supplementary Table S4), nor survival to 3 months (GLMM, binomial, $\chi^2_1 = 0.12$, $N = 131$ pups, $P = 0.72$). Relative fetus size (measured relative to other scanned females in a particular breeding attempt) also did not predict a female's share of total group reproductive success (GLMM binomial, $\chi^2_1 = 1.14$, $N = 153$, $P = 0.29$) nor pup survival to 3 months (GLMM binomial, $\chi^2_1 = 1.09$, $N = 131$, $P = 0.30$). Thus, we found no evidence that the production of larger fetuses translated into improved success in postnatal reproductive competition, at least in the short term.

Finally, we found no evidence of a cost of prenatal investment to mothers in terms of future survival or reproduction. In fact, higher total prenatal investment was associated with higher post-scan survival of mothers (Cox regression, Wald $\chi^2_1 = 6.57$, $N = 360$, $P = 0.010$; Figure 3). Again this relationship was driven by fetus size rather than number (Supplementary Table S5). Females that invested more prenatally were not less likely to reproduce in the next breeding attempt (GLMM binomial, $\chi^2_1 = 0.35$, $N = 164$, $P = 0.061$; Supplementary Table S6). Thus we found no evidence of a survival cost to mothers of elevated prenatal investment, nor did mothers compensate for high prenatal investment by reducing reproductive effort in the next breeding attempt.

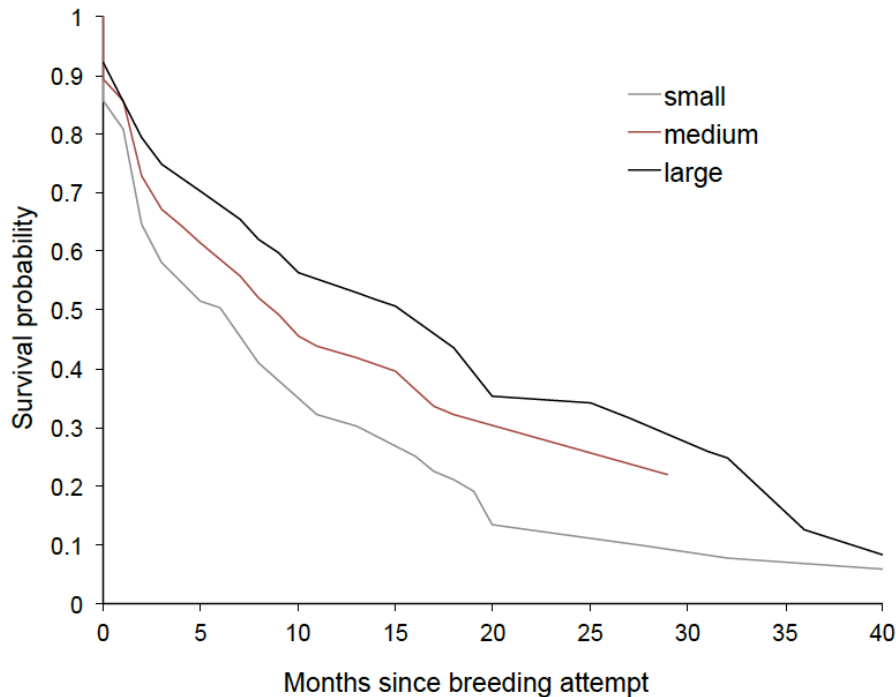


Figure 3. Maternal survival as a function of prenatal investment. Mothers that invested more prenatally survived longer. Fetus size (mean \pm sd =247.90 \pm 100.88mm²) has been categorized for illustrative purposes using the 25% (179.54mm²), mean and 75% (319.09mm²) quartiles.

Discussion

Female banded mongooses produced larger, but no fewer, offspring when there were more adult females in the group. Since all adult females breed in most breeding attempts, this is consistent with the hypothesis that females strategically up-regulate prenatal investment in the face of elevated postnatal reproductive competition. Such responses may be particularly likely to evolve in breeding systems where females co-breed regularly. Females showed steeper increases in prenatal investment when ecological conditions were harsh, and

when they were in relatively poor body condition, two factors which are expected to exacerbate the intensity of postnatal competition among offspring (Douhard *et al* 2014). We found no evidence that increased prenatal investment incurred future costs to females in terms of reproduction or survival. On the contrary, females that invested more prenatally showed improved future survival (Figure 3). A positive relationship between current reproductive investment and future survival is expected where females vary considerably in quality or access to resources, since high quality females may be able to divert more resources to offspring production without compromising their somatic function (the 'big house big car' effect, Van Noordwijk & Jong 1986; Resnick *et al* 2000).

Increasing fetus size in response to increased social competition is a subtle way that females could compete over reproduction within social groups without risking the costs of fighting or killing offspring (Clutton-Brock & Huchard 2013; Cant & Young 2014). However, we found no detectable benefit (in terms of short-term reproductive success) associated with increased investment in fetus size. Neither absolute fetus size nor fetus size relative to other co-breeders predicted the number of offspring that survived to emerge from the den. The lack of any detectable advantage to elevated prenatal investment is surprising, and may reflect a high level of noise associated with high pup mortality due to intra- or intergroup infanticide and predation (Cant *et al* 2013; 2014). It may also be that the benefits of increased prenatal investment are realised later in the life of the offspring. Studies of human famine and laboratory rodents, for example, suggest that early life environments can influence health and fitness across the lifespan, not just in the short term (Gluckman & Hanson 2004).

Our findings offer an interesting contrast to studies of social birds and fish, in which dominant females produce smaller eggs or a larger number of eggs when there are many helpers in the group (Russell *et al* 2007; Canestrari *et al* 2011; Taborsky *et al* 2007; Koenig *et al* 2009). In banded mongooses, all group members contribute to rearing young, but prenatal investment did not vary with the potential number of helpers (measured by total group size). Our findings suggest that the intensity of reproductive competition, rather than the availability of helpers, is the main determinant of variation in prenatal investment in this species. Larger pups have better access to adult group members who provide parental care and, upon emergence, aggressively defend access to the best helpers or 'escorts' (Gilchrist 2008). Where postnatal competition among offspring has characteristics of contest competition, the best response to competition will be to invest more resources per offspring prenatally, rather than to produce more of them (Parker 1974; Grinsted *et al* 2014). Producing a larger number of offspring could also bring benefits, but at the unavoidable cost of intensified competition among littermates.

Our study complements previous studies which suggest that mothers use hormones to influence the development of their offspring *in utero* to improve their success in the postnatal environment, a form of PAR (Gluckman & Hanson 2004; Bateson *et al* 2004). The PAR hypothesis has been criticized because long term forecasts of environmental conditions are inherently unreliable (Wells 2007; Rickard & Lummaa 2007). In cooperative breeders, however, the quality of the postnatal environment is largely determined by the number of breeders competing for reproduction and the number of helpers available to offspring. These features of social groups remain stable over the course of offspring

development, from gestation to nutritional independence, so are highly predictable. Cooperative birds and mammals, including humans, are thus likely candidates to evolve PARs. We found evidence that female banded mongooses respond to reproductive competition by adjusting prenatal investment, consistent with the PAR hypothesis, but we did not find evidence that this response is adaptive. Our study shows evidence that prenatal investment is altered in response to adverse social and environmental factors through lack of rainfall or low female weight at conception interacting with increased number of adult females in a group. To test the PAR hypothesis fully will require study of the consequences of variation in prenatal investment across the lifetime of offspring in animals exposed to natural predators and pathogens.

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Author contributions:

MC, EV and EI designed research; EI performed research; EI, FT HM and EV analysed data; HN and JS carried out genetic analysis; EI, MC and EV drafted the paper; all authors contributed to the final version.

Chapter 3: Patterns of spontaneous abortion in female banded mongooses

Abstract

Female reproductive competition occurs when two or more females compete for limited resources needed for reproduction. The intensity of reproductive competition can determine whether the fitness benefits of current reproductive effort exceed the potential costs to survival and future fertility. In social species, spontaneous abortion could be a response to reproductive competition, permitting females to curtail reproductive expenditure on offspring that are unlikely to survive in competition with the offspring of co-breeders. We tested this hypothesis using long-term data on banded mongooses, in which multiple females within a group give birth synchronously to a communal litter that is cared for by the group as a whole. We found that young females were more likely to abort than older females in a given breeding attempt, and that the probability of abortion decreased with increasing rainfall (a proxy for insect prey abundance). We did not find evidence that females were more likely to abort in asynchronous breeding attempts, or when there were a larger number of female breeders. Our results suggest that abortion may be a means by which young, otherwise healthy females conserve resources for future breeding attempts in more benign conditions.

Keywords: banded mongoose, abortion, female reproductive competition, cooperative species, reproductive suppression

Introduction

Female reproductive competition can be just as intense as male-male competition, but this is easily overlooked as female competitive strategies can be subtle (Stockley & Bro-Jørgensen 2011). Females compete over access to and control of resources (which can include access to mates) essential to the survival and reproductive success of their offspring; which determines a female's own reproductive success. In reindeer (*Rangifer tarandus*), for example, dominant females monopolize access to superior foraging patches, gain body mass during the winter, and consequently breed earlier and more often than subordinate females, and produce calves that grow faster (Holland *et al* 2004). In high reproductive skew societies like the common marmoset (*Callithrix jacchus*), a cooperative breeder, dominant females and other non-breeding subordinate females will aggressively suppress subordinate female reproduction through infanticide (Saltzman *et al* 2009; Creel & Creel 1991).

One of the subtle consequences of female reproductive competition may be female spontaneous abortion.

Spontaneous abortion of a pregnancy in response to social cues has been observed in many mammals, particularly rodents in which females will abort or reabsorb fetuses in the presence of a new unknown male ('The Bruce effect', Bruce 1959; Mahady & Wolff 2002; Roberts *et al* 2012). This appears to be an adaptive strategy in conserving a female's time and resources to raise future offspring with better survival probabilities, than a current litter likely to fail (Stearns 1992). However, in systems where multiple females compete for reproduction, it can be as a result of aggression, the threat of eviction from the

group (risking the females own survival), or infanticide from dominant females reducing an individual's probability of breeding successfully. To avoid being evicted or infanticide, females can either suppress or abort reproduction in favour of helping and remaining in the group and waiting for a better future opportunity to breed (Saltzman *et al* 2009; Gilchrist *et al* 2006).

In this study, we tested the role of reproductive competition as a predictor of abortion in a population of wild banded mongooses (*Mungos mungo*). Banded mongooses provide a useful study system for looking at effects of female competition, as multiple females within a social group reproduce at the same time, typically giving birth on the same day. The combined group litter is cared for by all the adults and pups compete over access to helpers (Cant *et al*. 2013). Female competition and number of females increases female investment in fetus size and ultimately effects through pup size and competition (Gilchrist 2008; Hodge *et al* 2009; Inzani *et al* 2016). We predicted that abortion should be more frequent in (1) asynchronous breeding attempts, and (2) in breeding attempts where there were many pregnant females. Within breeding attempts, we predicted (3) that younger females and those in poor condition should show an elevated risk of spontaneous abortion.

Methods

Study population

This study uses data collected from a population of banded mongooses living on and around Mweya Peninsula, QENP, Uganda (0°12'S, 27°54'E) between September 1999-February 2015. For a detailed description of the climate, habitat and the population see Cant *et al* 2013. All individuals in the population are individually marked and captured, and each individual is visibly identifiable from unique shave marks (for details of capture and anaesthesia see Jordan *et al* 2010). Each group is visited every day to determine accurate parturition and abortion dates. Pregnancy is determined from observations in changes in body shape, and upon routine capture, from ultrasound scans and palpation of the abdomen. A previous study found no adverse effects of trapping and anaesthesia on pregnant females (Gilchrist *et al* 2004). Parturition was determined from sudden change of abdomen size, coupled with the observation of temporary disappearance of adult individuals due to baby sitting in the den. Abortion was distinguished from parturition by the difference in the length of time elapsed between the end of observed oestrus for the females in the group and sudden reduction in abdomen size. Abortions were defined as confirmed pregnancies where the length of gestation was less than the average 60 days (average from the mean period between peak mate guarding and birth; Cant 2000) and where babysitting behaviour was not observed. There were also direct observations (N =9) from the individual females where unviable neonates/blood on vagina was seen.

For each breeding attempt, pregnant females were scored as to whether they aborted (yes/no) and whether they were evicted from the group. Females were defined as having been evicted if they left the group for at least 24hrs as a result aggression from other group members (see Thompson *et al* 2016 for further details of eviction behaviour). For this study we were interested in spontaneous abortions that might serve to avoid reproductive competition, rather than abortions that may have resulted from direct aggression from other females; hence breeding events in which an eviction occurred were excluded from the analyses.

We tested two measures of breeding asynchrony. First, we used the duration of 'group oestrus' as a measure of breeding asynchrony between the females giving birth within a group, with longer group oestrus indicating higher asynchrony between females and their reproductive cycles. The duration of group oestrus was calculated as the number of days from the first observed mating or mate-guarding behaviour to the last observed mating or mate-guarding behaviour in the group. We also recorded the number of days that it took for all the reproductively active females to give birth for each group breeding attempt, the length of time again used as a proxy for litter asynchrony.

To test if females are aborting due to lack of resources available to be invested in viable fetuses, we measured fetus size using a portable ultrasound scanner. Fetus size was estimated from cross-sectional areas of foetuses from individual ultrasound scans (Details of methods in Inzani *et al* 2016). Female weight at the time of conception was calculated using the closest weighing event prior to the estimated conception date.

Group size and the number of females were counted as the total number of adult individuals (≥ 10 months old) in each group for each communal litter. Rainfall data was provided by Mweya metrological station. All research was conducted under permissions from Uganda Wildlife Authority (UWA) and Uganda National Council for Science and Technology (UNCST), and methods approved by the ethical review panel of the University of Exeter.

Statistics

Factors influencing individual females and groups

To test what the likelihood of abortion within a group breeding attempt, or with individual females, we used generalised linear mixed models (GLMMs) with binomial distribution (link="logit") in R (R Core development team, R version 3.1.0 (2014); lmer package Bates *et al* 2014). To account for repeated sampling across groups and individuals, group was included as random factor in all analyses, and female identity and litter in the individual level analyses.

To test what affected the probability of an abortion occurring in a breeding event, we used GLMM with the sum total rainfall during pregnancy (from conception to the birth of the group litter; ml), number of adult females (> 1 year old) present in the group, number of adult males (> 1 year old) and total group size, the group duration of oestrus (number of days between the first and last observation of mating in the group) and the group parturition asynchrony (number of days between the first and last female in a group to give birth) as fixed factors.

To investigate what affected a particular female's probability of aborting in a

given litter, we included females from breeding events where an abortion had occurred. Fixed terms included were: female weight at conception (g), female age (years), number of adult females present in the group, group size, mean fetus size (mm^2), and total rainfall during pregnancy (ml); with interaction terms between rainfall*number of females, rainfall*female age, female age*female weight and number of females*female age. Because groups were trapped at different stages of pregnancy, fetus age was included as a covariate together with fetus size.

Testing factors with high covariance (>0.5 , shown to cause problems in model fitting Freckleton 2011), like group size and the number of females present were tested independently from each other. The measures of breeding asynchrony between females or factors that limited sample size dramatically (fetus size) were tested in separate models (Tables of each model is in the Supplementary Information). A minimal model was obtained from sequential removal of least significant factors, starting with 2-way interactions. Each factor was then added back into the minimum model in order to confirm removal was not contingent on the order of removal.

Results

Summary of data:

In 133 out of 461 recorded group breeding attempts, at least one female aborted prior to birth. In these 133 breeding attempts, the mean (\pm S.D.) number of females to abort was 2.06 ± 1.26 , equating to about 36% of the females that participated in a single group breeding attempt. From 207 observed individual female pregnancies, 74 (36%) were not carried to term in which the majority

occurred during the 1st trimester and early 2nd trimester (between days 0 and 25).

What predicts the likelihood of an abortion occurring within a breeding attempt?

Groups were more likely to have at least one female abort when rainfall was low (binomial GLMM: Rainfall, $\chi^2=7.58$, N =436, P =0.006; Figure 1). None of the other tested variables - measures of female asynchrony (oestrus duration and birth asynchrony), number of adult females present in a group, nor the total group size – predicted occurrence of abortions among litters (Supplementary Information, Table 1).

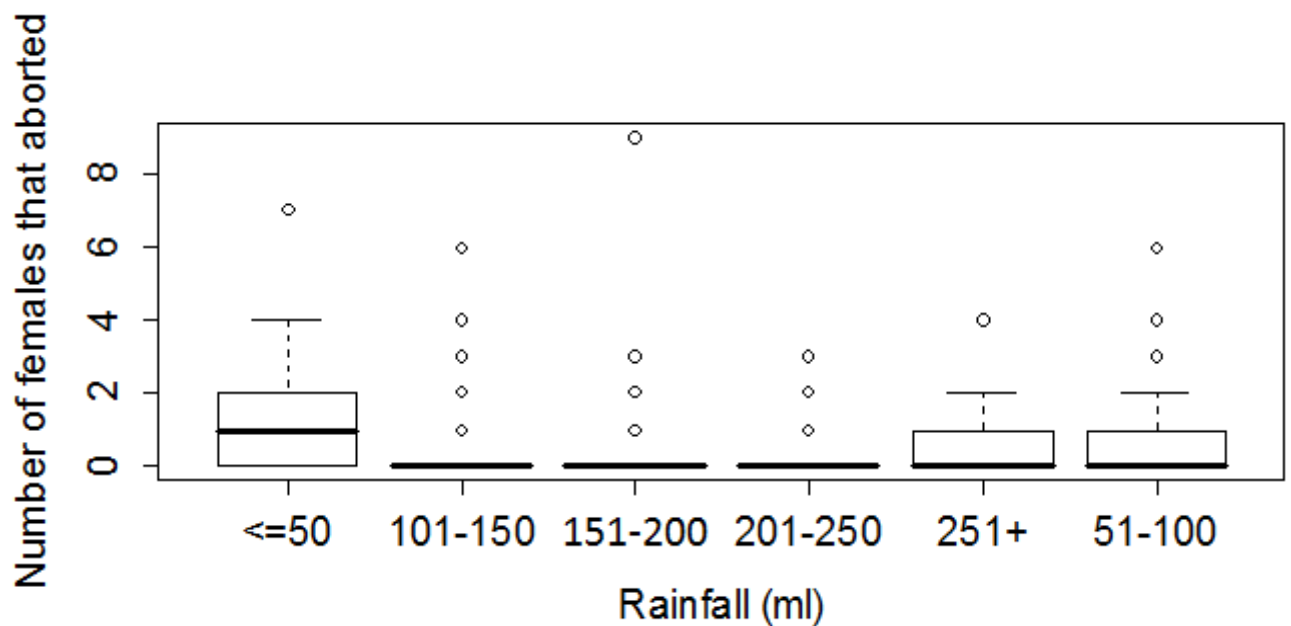


Figure 1: Probability of abortion within a breeding attempt decreased with increasing rainfall

Within a breeding attempt, what predicts a female's probability of aborting a litter?

Young females were more likely to abort (binomial GLMM, Female age, $\chi^2=9.54$, $N=207$, $P=0.002$) but neither female weight at conception, rainfall, number of females present, nor group size (or any of the interaction terms included) were significant ($N=207$, $P>0.05$, Supplementary Information Table 2, Figure 2). Fetus size also did not influence the probability of an individual female aborting in a given litter (binomial GLMM, $\chi^2=2.06$, $N=76$, $P=0.15$).

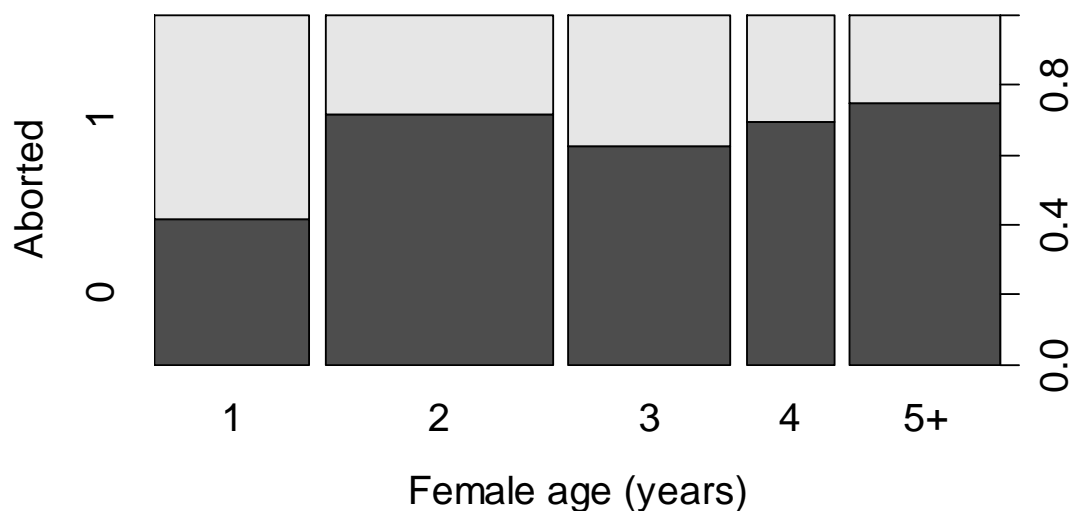


Figure 2: Proportion of females that aborted their pregnancy grouped into age classes for illustrative purposes; age was treated as continuous variable in all models.

Discussion

Female banded mongooses were more likely to spontaneously lose their pregnancy if they were young, with an abortion within a group being more likely to occur during periods of low rainfall. Unlike other studies into female reproductive success, we found no effect of female weight at conception (potential indicator of a female's condition) on whether a female was likely to abort or carry to term (Wauters & Dhondt 1989; Cameron *et al* 1993). We also found no effect of fetus size as to whether a female would abort or not. Therefore these results imply that the females were in good enough condition to breed and the fetuses measured were viable to gestate to term. Suggesting any subsequent abortions were as a result of environmental or social conditions. Low rainfall throughout pregnancy was associated with an increased likelihood of abortion within a group. Rainfall is strongly correlated with insect prey abundance (Marshall *et al* 2016), and hence low rainfall may provide a cue that pups are likely to face increased post-natal competition for food and access to helpers (Gilchrist 2008; Hodge *et al* 2009).

From our study, it appears that females are likely to spontaneously abort when the possibility of reproductive failure is high due to adverse environmental conditions. Under such unfavourable conditions, when resources are scarce and offspring survival limited, it may be beneficial for a female to abort her pregnancy in favour for a future breeding attempt. This is particularly true for younger females that may lose out in any competition, as older females are tend to be more dominant, and will harass subordinate females (and can evict females from the group) or kill subordinate females' offspring in order to reduce

competition (Cant *et al* 2014; Gilchrist *et al* 2006). Breeding while young can come at a higher cost than when you are older as breeding too early can compromise female survival, future reproduction; young females are also more inexperienced (Hanssen *et al* 2005; Côté *et al* 2001; Reid *et al* 2003). This suggests that it would be a better strategy to conserve energy and cut losses and reproduce later (Stearns 1992).

Stress is also known to play a key role in the maintenance of pregnancy and whether females can carry to term. In meerkats it has been suggested that increased stress measured by faecal glucocorticoids in subordinate females from prolonged aggression and possibly eviction, caused females to abort or reduced their condition so that they were unable to reproduce (Young *et al* 2006; Gilchrist *et al* 2006). Further research would be required to examine further whether it is the levels of stress experienced during pregnancy in banded mongooses also influences whether females gestate to term, and how this might affect their own and their offspring's fitness. In this study, breeding events in which violent evictions were excluded, but subtler forms of aggression can still suppress subordinate reproduction. As seen in naked mole rats (*Heterocephalus glaber*), where the dominant female inhibits reproduction in her subordinates through frequent, aggressive interactions (Lacey & Sherman 1997). Overall, spontaneous abortion in banded mongoose females may be an adaptive means to escape potentially costly female reproductive conflict.

Chapter 4: General Discussion

Overview of findings

Females can experience strong reproductive competition in producing offspring. Although the means of such competition are often subtle, it is particularly noticeable in cooperative breeders. As seen in banded mongooses (*Mungos mungo*), reproductive suppression is not just direct aggression towards the females themselves (with the threat of eviction from the group). It can also be an implied indirect threat of infanticide, reducing potential reproductive competitor's reproductive success. The ability of a female to adapt her prenatal investment according to current or potentially future conditions has far reaching implications for her offspring's growth and survival, but also for that of her lifetime reproductive success. This is particularly important when postnatal competition between offspring of different females is intense, and females have a limited ability to boost their own offspring's fitness after birth. Early female reproductive investment is better studied in birds rather than mammals, due to the obvious difficulties in quantifying investment in fetuses versus eggs, especially within wild populations. This means that the factors affecting prenatal investment in relation to reproductive conflict and its consequences in mammals are still relatively unknown. There are even fewer data available on the spontaneous loss of pregnancy and reproductive competition; with the exception of studies on microtine rodents. It is currently unknown to what extent and why spontaneous abortions occur in cooperatively breeding species and how this affects female fitness. These data are difficult to gather, yet important

in order to fill the gaps and help increase our understanding of how prenatal investment can be altered in response to resource limitations and competition.

To help fill this gap in our knowledge, I have investigated the factors affecting and the potential consequences of prenatal investment in a cooperative breeder, the banded mongoose. This study is one of the first to use ultrasound scans to measure fetus size fetal litter size in wild cooperatively breeding mammal. This study shows that using cross-sectional ultrasound scans of the fetus allows a precise and repeatable measure of the cross-sectional area of the fetus, as a proxy for foetus size, which in combination with foetal litter size allows for quantifying individual female's investment into current reproduction. Competition with other females also affected prenatal investment, with females increasing their investment in response to more of them breeding together. This effect was particularly visible in harsh conditions, when rainfall was low or females were light in weight at conception. Surprisingly, I found no benefits to this increase in investment for individual pups or their mothers, which may be due to other stochastic factors such as predation having a stronger influence on pup survival. Using data from confirmed pregnancies, I was also able to quantify the proportion of pregnancies that were not carried to full term, and to investigate factors influencing spontaneous abortion in banded mongooses. In short, pregnancy loss was more likely in adverse environments and younger and socially lower ranking females were more likely to abort overall. Below, I discuss the key findings of this thesis, how they fit with our current knowledge of female reproductive conflict, and suggest areas where future research would help further our understanding.

Prenatal investment and female reproductive conflict

Female reproductive competition is commonplace and seen in many species (Chapter 1). However, its effect on prenatal investment is almost completely unknown in wild mammals due to the difficulties in assessing investment during gestation. Understanding how female reproductive conflict occurs within groups and what the effects on individual's prenatal investment is important as it can have wider implications for the female and her offspring's future survival and reproduction (Stockley and Bro-Jørgensen 2011). In Chapter 2, I found evidence that female banded mongooses are altering their prenatal investment in response to increased competition for resources. When the number of co-breeding females increased, female investment in fetus size also increased. The effect seen of increasing fetus size increased further if rainfall was high or females weighed more as the amount of resources available that could be allocated to reproduction had increased. However, it was the lighter weight females or in periods of low rainfall that females showed the largest response to increasing female competition. Increased female reproductive competition due to lack of resources available also indicates that the postnatal competition between offspring is also likely to be intense.

Females did not appear to be competing directly for the resources in order to breed; but to try and potentially give their pups a competitive advantage against the rest of the cohort. (Gilchrist *et al* 2008; Hodge *et al* 2009). However, this study found no evidence that increased investment in fetuses resulted in improve offspring success, at least in the short term. Pups were not significantly different in weight or survival to 3 months. This could be because of other

factors impacting pup survival postnatally that have overriding effects, such as the stochastic effect of predation. It could also be that the benefits to increased prenatal investment influence the offspring later in life, in their survival and reproductive success (Reid *et al* 2003; Gorman & Nager 2004; Van de Pol *et al* 2006). Another possibility is that the beneficial effects of increased prenatal investment were masked by the fact that all the females within a breeding attempt would up-regulate their individual investments in response to increased reproductive and future postnatal competition (A kind of “Red Queen” effect, Kauffman 1995). This would require an experiment to manipulate female prenatal investment of some females in order to try and uncover any potential impacts this may have on their pups.

While I did not find any strong evidence for or against any adaptive benefit to increasing prenatal investment between females, there was a trend of increasing investment in size with female lifespan. This suggests that females who are better in condition/plenty of resources they can allocate more resources to their reproductive effort without compromising their own survival. In reindeer (*Rangifer tarandus*), females that lived longer, did raise more young successfully because, not just because they had more opportunities to breed and females had more experience in rearing calves but also because longevity correlated with female condition/quality (Weladji *et al* 2006; Holland *et al* 2004).

There was also no evidence of a quantity/quality trade-off. This is probably due to that females only produce the number of offspring that they can viable gestate to term and can partially reduce their prenatal litter through reabsorption if conditions deteriorate (Pratt & Lisk 1989). If females are of high quality with

lots of resources available to them, they can also produce more and larger fetuses without a cost to their survival, following a 'Big house, big car' scenario (Reznick *et al* 2000, Van Noordwijk & de Jong 1986).

Abortion and female reproductive conflict

Failure to breed successfully is an important and common occurrence, yet the reasons behind spontaneous abortions remain little studied. In the banded mongooses, spontaneous abortions were observed in 74 out of 207 breeding events where I had data on confirmed pregnancies (Chapter 3). Overall, abortions were more likely to occur during times of low resource availability, which indicates female condition may play a part in spontaneous pregnancy loss.

The only significant predictor of a given female aborting was her age, with younger females more likely to abort, and female weight was not a significant predictor of abortion. There were no interactions with resource abundance (rainfall) either or potential reproductive competition (number of females in a group). Female banded mongooses did not appear to be aborting their pregnancies as a result of lack of resources. Low rainfall did make it more likely that a spontaneous abortion would occur in a group, but it also indicates that reproductive competition between females is likely to be stronger. As younger females are likely to be of lower ranking, implying it could be due reproductive suppression from dominant females due to subtle low levels of aggression/threat (Cant *et al* 2010; Cant *et al* 2014). This could make

spontaneous pregnancy loss be an adaptive strategy for females to conserve their energy and time for the future when the competition has decreased.

Young females are also more inexperienced breeders, and potentially more likely to be out of synchrony with the other breeding females when reproducing for the first time (Gilchrist *et al* 2006). Young females could be induced to abort their current breeding attempt, to conserve resources for a future attempt that is more likely to be successful. Breeding while young can come at a higher cost than when you are older as breeding too early can compromise female survival, future reproduction; young females are also more inexperienced (Hanssen *et al* 2005; Côté *et al* 2001; Reid *et al* 2003). This suggests that it would be a better strategy to save resources for future opportunities (Stearns 1989). However, this study found no evidence for increasing asynchrony affecting the likelihood of a female aborting. Our measures of asynchrony (the duration in days that females within a group were in oestrus and the duration in days they gave birth on), are likely to be too broad to pick up the fine-scale differences between individual female asynchrony. Females are also not likely to be comparing their exact synchrony against that of the rest of the group because it is not a simple or reliable measure. Yet their relative age and rank in the group, along with the levels of rainfall will be known to them, allowing a much better estimation of the relative competition they and their offspring may experience.

Conclusions/future research

From my findings in chapters 2 and 3, it seems that females can maximise their fitness in a highly competitive environment through two strategies: either to abort the current attempt in favour of better future conditions, or to attempt to make a success of the current breeding opportunity, altering their investment to best match the short-term environment if possible. Previous studies have not been able to investigate the implications of female reproductive conflict on prenatal investment in wild mammals, yet it can have important consequences for the future of their offspring as well as their own fitness.

Further investigations are needed into female variation in prenatal investment in wild mammals and its consequences for pup growth and survival. An aim arising from the work presented in this thesis is to investigate this further in the banded mongoose. With expansion of the detailed pedigree the project has on the population, it would be possible to examine more individuals, of known prenatal investment from their mother, to investigate how this influences their own life histories and reproductive success.

Prenatal investment may also take different forms if some females are predisposed to alter the sex ratio of the fetuses she produces according to varying costs and benefits to produce offspring of different sex (Clout *et al* 2002). Currently, data on sex ratios of litters rely on data after litter emergence, typically up to 4 weeks after the pups are born. With recent advances in technology it would also be possible to use a more detailed ultrasound scanner to build on the findings of this thesis, as it should be possible to determine the

sex of each fetus and the sex ratios of each litter in utero, to compare composition to the group litter of pups seen on emergence.

A more detailed scanner would also allow the earlier detection of pregnancy (that would otherwise not be noticeable until later in gestation), allowing further investigation in the number of fetuses a female gestates and possible early abortion/re-absorption of all or some of the fetuses carried in response to environmental conditions and female reproductive competition. Currently, studies that have looked at this have been done on microtine rodents using destructive sampling of individuals, which would not be viable in long-term studies including detailed information on life-history of individuals (Malhady & Wolff 2002). Adopting and further developing the non-invasive sampling described in this thesis has the potential to further increase our understanding of female reproductive dynamics in the wild, and effects of prenatal investment on female fitness and ultimately life-history evolution.

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Appendix

Supplementary material

Table S1: Factors affecting prenatal investment

Model terms	Fetus cross-sectional area (fetus size)					Number of fetuses					Total prenatal investment (fetus size x number of fetuses)				
	Effect size \pm SE			χ^2	<i>P</i>	Effect size \pm SE			χ^2	<i>P</i>	Effect size \pm SE			χ^2	<i>P</i>
Female age (months)	-0.12	\pm	0.38	0.10	0.75	0.12	\pm	0.038	10.36	0.0013	1.8	\pm	1.5	1.34	0.25
Female age ² (months)	-0.0018	\pm	0.0042	0.087	0.77	-0.053	\pm	0.025	4.81	0.028	0.021	\pm	0.016	1.56	0.21
Female weight (g)	0.30	\pm	0.10			0.036	\pm	0.033	1.19	0.27	0.58	\pm	0.16	12.60	<0.001
Rainfall during pregnancy (ml)	1.81	\pm	0.70			-0.0079	\pm	0.031	0.068	0.80	-0.68	\pm	0.85	0.64	0.42
Number of females	-53	\pm	57			-0.0094	\pm	0.031	0.09	0.76	38	\pm	15	5.65	0.017
Female weight x number of females	-0.029	\pm	0.014	4.23	0.040										
Total rainfall x number of females	-0.24	\pm	0.11	4.91	0.027										
Group size	1.8	\pm	2.6	0.089	0.77	0.0012	\pm	0.03	0.0015	0.97	-9.9	\pm	10	0.85	0.36
Fetus age (days)	-45	\pm	20												
Fetus age ² (days)	0.64	\pm	0.29												
Sample	360 ultrasounds from 59 females in 41 litters from 8 groups.					361 observations from 127 females in 130 litters from 11 groups					360 ultrasounds from 59 females in 41 litters from 8 groups.				

Random effects: female ID, litter ID and group ID. Model terms were scaled in GLMM analysis on number of fetuses.

Table S2: Within- and between- female variation in fetus size

Model terms	Effect size	±	SE	χ^2	P
Female age (months)	0.78	±	0.35	0.045	0.83
Female age ² (months)	0.00061	±	0.0041	0.021	0.89
Female weight at conception (g)	0.064	±	0.043	2.00	0.16
Rainfall during pregnancy (ml)	-0.094	±	0.23	0.17	0.68
Within-female effects	12.24	±	5.63	4.51	0.034
Between-female effects	9.55	±	4.76	3.38	0.066
Fetus age (days)	2.88	±	1.20		
Fetus age ² (days)	-0.048	±	0.093		
Sample	360 ultrasounds from 59 females in 41 litters from 8 groups.				

Random effects: female ID, litter ID and group ID.

Table S3: Consequences of prenatal investment – female reproductive success

Model terms	Number of emergent pups assigned to female					Proportion of pups in a group litter assigned to female				
	Effect size ± SE			χ^2	P	Effect size ± SE			χ^2	P
Mean fetus size (mm ²)	0.0022	±	0.0017	1.66	0.20					
Relative fetus size						0.0023	±	0.0022	1.14	0.29
Number of fetuses	0.28	±	0.12	5.44	0.020					
Fetus age (days)	0.05	±	0.03			0.0025	±	0.0017		
Sample	153 observations from 78 females in 51 litters from 10 groups.					153 observations from 78 females in 51 litters from 10 groups.				

Random effects: female ID, litter ID and group ID.

Table S4: Consequences of prenatal investment – Pup survival and growth to independence

Model terms	Pup survival to 3 months					Pup growth (age≤90 days)				
	Effect size ± SE			χ^2	<i>P</i>	Effect size ± SE			χ^2	<i>P</i>
Mean fetus size (mm ²)	0.0014	±	0.0041	0.12	0.72	0.04	±	0.06	0.34	0.56
Relative fetus size (mm ²)	0.0063	±	0.0023	1.09	0.30					
Number of fetuses	0.02	±	0.27	0.0058	0.94	-7.0	±	5.5	1.59	0.21
Total number of pups in a group litter	-0.05	±	0.10	0.23	0.63	-0.94	±	1.55	0.37	0.54
Sex of pup						9.68	±	10.30	0.87	0.35
Pup age (days)						4.0	±	1.1		
Fetus age (days)	0.02	±	0.06			4.1	±	1.0		
Sample	131 pups from 29 litters from 8 groups.					116 pups from 26 litters from 8 groups.				

Random effects: litter ID and group ID.

Table S5: Consequences of prenatal investment– female survival

Female post-reproductive survival (months). Cox regression with backward selection of terms (Wald).

Model terms	Effect size	±	SE	Wald χ^2	P
Number of females	- 0.081	±	0.0052	2.23	0.14
Total prenatal investment	-0.001	±	0.0001	6.57	0.010
Number of fetuses	-0.009	±	0.121	0.006	0.94
Mean fetus size (mm²)	-0.005	±	0.001	12.68	<0.001
Relative fetus size (mm ²)	0.001	±	0.003	0.16	0.69
Sample	109 females in 47 litters from 10 groups.				

Table S6: Consequences of prenatal investment – female participation in next litter (y/n)

Model terms	Effect size	±	SE	χ^2	P
Female age (months)	-0.01	±	0.02	0.17	0.68
Female age ² (months)	-0.0015	±	0.0029	0.28	0.60
Female weight at conception (g)	0	±	0.0031	0.012	0.91
Number of females	0.19	±	0.48	0.16	0.69
Mean fetus size (mm ²)	-0.0012	±	0.0072	0.030	0.86
Number of fetuses	-0.87	±	0.52	3.50	0.061
Fetus age (days)	0.06	±	0.14		
Sample	105 observations from 46 females in 34 litters from 7 groups.				

Random effects: female ID, litter ID and group ID.

Supplementary Information

Table S1: Factors effecting pregnancy loss in group breeding attempts

Model terms	Effect size	±	SE	χ^2	P
Rainfall ^a	-0.0044	±	0.0017	7.58	0.006**
Number of females ^a	0.035	±	0.051	1.85	0.17
Total group size ^a †	0.025	±	0.016	2.46	0.12
Group oestrus duration (days) ^b	-0.078	±	0.055	2.11	0.15
Group birth asynchrony (days) ^c	-0.0090	±	0.032	0.08	0.78
Sample	^a 436 group breeding attempts from 18 groups. † Group size was tested in a model with rainfall separately from the number of females and males due to high correlation between the factors. ^b 439 group breeding attempts from 18 groups. ^c 105 group breeding attempts from 10 groups.				

Random effects: group ID.

Table S2: Factors effecting individual female pregnancy loss

Model terms	Effect size	±	SE	χ^2	P
Rainfall (ml)*female age (days)	-0.0012	±	0.0011	1.19	0.28
Rainfall (ml)*Number of females	-0.00039	±	0.00040	0.12	2.27
Female age (days)*Number of females	0.066	±	0.0031	0.16	0.69
Female age (days)* Female weight at conception (g)	0.00065	±	0.00011	0.15	0.70
Rainfall (ml)	-0.0022	±	0.0027	1.19	0.28
Number of females	-0.11	±	0.095	1.97	0.16
Average fetus size (cross sectional area mm ²) †	-0.0037	±	0.0035	2.06	0.15
Female age (days)	-0.30	±	0.12	9.54	0.002**
Female weight at conception (g)	-0.00035	±	0.0010	0.08	0.78
Sample	207 individual female breeding attempts from 93 individuals, 57 litters (group breeding attempts) in 8 groups. † Fetus size was tested in a model with the age of the fetus at the time of the ultrasounds scan separately due to the small sample size; n=76 from 41 individuals from 17 litters in 6 packs.				

Random effects: Individual ID, breeding attempt ID, group ID.